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(54) Abstract Title

A synergistic composition comprising mussel protein extract and glycosaminoglycan suitable for treatment of arthritis

(57) A pharmaceutical composition comprising proteins extracted from the New Zealand green-lipped mussel (Perna canaliculus) and one or more glycosaminoglycans, preferably glucosamine or glucosamine sulphate, has useful anti-inflammatory properties. This composition may be used in the treatment of arthritis conditions, such as osteoarthritis or rheumatoid arthritis. The combination of the protein extract and the glycosaminoglycan is synergistic with respect to the effect of the same concentration of the individual components of the combination. A preferred composition includes a homogeneous mixture of a freeze-dried powder containing proteins extracted from the mussel (about 66.6 wt%) and a glycosaminoglycan powder (about 33.3 wt%). A method for preparing this composition is described. The composition may be administered in capsule or tablet form.

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MUSSEL/GLYCOSAMINOGLYCAN SYNERGISTIC COMPOSITIONS AND USE

This invention relates to synergistic anti-inflammatory compositions, the methods of producing them, and the methods of using them.

Anti-inflammatory compositions are useful in the treatment of arthritis, such as rheumatoid arthritis and osteoarthritis, to provide symptomatic relief. The New Zealand green-lipped mussel (*Perna canaliculus*) is known to contain substances having a beneficial anti-inflammatory effect, making it useful in the treatment of arthritis.

In our New Zealand patent application No. 328489 we disclosed an invention relating to an anti-inflammatory composition including a freeze-dried substance containing proteins, including glycogen, extracted from the New Zealand green-lipped mussel.

One aspect of that invention consisted in a method of preparation of an anti-inflammatory composition comprising:

- homogenising flesh of the New Zealand green-lipped mussel in approximately ten volumes of approximately 45% aqueous phenol;
- stirring the homogenised flesh for approximately 45 minutes at about 20°C;
- centrifuging the mixture for approximately 10 minutes at substantially 250g;
- aspirating the upper aqueous layer (containing the glycogen product);
- precipitating the glycogen product with about three volumes of approximately 95% ethanol;
- isolating the product by centrifugation for about 15 minutes at approximately 2300g;

- resuspending the product in the minimum amount of distilled water;
- reprecipitating the product;
- dialysing the suspension against distilled water for approximately 24 hours; and
- freeze drying

to yield a solid anti-inflammatory composition.

Another aspect of that earlier invention consisted in a method of preparation of a solid anti-inflammatory composition wherein the anti-inflammatory composition is separated from homogenised New Zealand green-lipped mussel flesh by chemical means.

Yet another aspect of that invention consisted in the use of an anti-inflammatory agent when prepared by the above methods.

In a preferred form of one aspect of that earlier invention, green-lipped mussel flesh was homogenised in ten volumes of 45% aqueous phenol and stirred for 45 minutes at 20°C. The preparation was then centrifuged at 250g for 10 minutes and the upper aqueous layer containing glycogen aspirated. Glycogen was precipitated with three volumes of 95% ethanol and isolated by centrifugation at 2300g for 15 minutes. The pellet was resuspended in a minimum amount of distilled water and the glycogen reprecipitated twice more. The final suspension was dialysed against distilled water for 24 hours, before being freeze-dried and stored at 4°C.

The resulting substance exhibits an anti-inflammatory effect when administered parenterally or orally in humans and animals.

Glycosaminoglycans are also known to have an anti-inflammatory effect.

It has been found that the combination of the substance containing proteins extracted from the green-lipped mussel and a glycosaminoglycan, particularly glucosamine sulphate or other pharmaceutically acceptable glucosamine salt, has a complementary effect wherein the degree of anti-inflammatory relief exceeds that which would be expected from the use of the relative concentrations of either ingredient alone.

The product containing the substance extracted from the green-lipped mussel possesses chondroprotective, gastroprotective and anti-inflammatory activity and is therefore beneficial to sufferers of many of the arthritic disorders. We believe that the chondroprotective activity is due to the presence of glycosaminoglycans (ca 4-6%), such activity resulting from the intermolecular binding properties of glycosaminoglycans, complemented by their hydrophilicity, or affinity for water molecules. Due to the polarised nature of glycosaminoglycans, the molecules tend to repel each other and consequently take up a large volume of molecular space. In addition the combination of glycosaminoglycans with naturally present water molecules results in large molecular complexes. We believe that glycosaminoglycans provide a buffering effect in joints, and help to increase the viscosity of the joint-lubricating fluids.

When the small amount of active anti-inflammatory substance in the material extracted from the green-lipped mussel is complemented by one or more glycosaminoglycans such as glucosamine sulphate or other pharmaceutically acceptable salt, the synergistic effect on chondroprotection complemented by its anti-inflammatory activity, provides unexpectedly increased relief for sufferers of arthritis.

In a first aspect, the present invention consists in a synergistic anti-inflammatory composition including a substance containing proteins extracted from the New Zealand green-lipped mussel (*Perna canaliculus*) and one or more glycosaminoglycans.

The proteins may be extracted from processed mussel tissue.

Preferably, the processed mussel tissue comprises ground mussel tissue.

Preferably, the processed mussel tissue is processed to concentrate the proteins.

Preferably, the substance containing proteins extracted from the green-lipped mussel is dried.

Preferably, the substance containing proteins extracted from the green-lipped mussel is in the form of a powder.

The glycosaminoglycan may be glucosamine or a pharmaceutically acceptable salt thereof, preferably glucosamine sulphate, chondroitin sulphate or N-acetyl glucosamine.

Preferably, the composition is prepared as a capsule wherein the anti-inflammatory composition is encapsulated in gelatine.

The synergistic anti-inflammatory composition may be prepared in the form of a tablet.

The invention also consists in another aspect in a method of treating inflammation in non-human animals by the administration of the synergistic anti-inflammatory compositions described above.

Preferably the administration is by administration of the synergistic composition in the form of a capsule.

Alternatively, the administration is by administration of the synergistic composition in the form of a tablet.

The above gives a broad description of the present invention, one preferred form of which will now be described.

In a preferred form of the present invention, about 66.6 wt% of freeze-dried powder containing proteins extracted from the green-lipped mussel, and about 33.3 wt% of a glycosaminoglycan powder, are blended together to produce a homogeneous mixture. This mixture may then be used to make capsules or tablets as desired.

The above freeze-dried material from the green-lipped mussel is prepared by:

- homogenising flesh of the New Zealand green-lipped mussel in approximately ten volumes of approximately 45% aqueous phenol;
- stirring the homogenised flesh for approximately 45 minutes at about 20°C;
- centrifuging the mixture for approximately 10 minutes at substantially 250g;
- aspirating the upper aqueous layer (containing the protein-containing product;
- precipitating the protein-containing product with about three volumes of approximately 95% ethanol;
- isolating the product by centrifugation for about 15 minutes at approximately 2300g;
- resuspending the product in the minimum amount of distilled water;
- reprecipitating the product;
- dialysing the suspension against distilled water for approximately 24 hours; and
- freeze drying

to yield the solid anti-inflammatory substance which may then be milled to produce the product in the form of a powder.

The mussel extract powder may be prepared by freeze drying the fluid extract derived by centrifuge from fresh, live mussels.

The glycosaminoglycan may be in the form of a pharmaceutically acceptable grade of glucosamine sulphate, chondroitin sulphate, N-acetyl glucosamine, or may be a mixed marine glycosaminoglycan complex.

Glucosamine sulphate, chondroitin sulphate or N-acetyl glucosamine may be obtained from commercial sources.

Marine glycosaminoglycans may be prepared from shark cartilage, or other suitable marine-based raw material, by known enzyme digestion processes followed by freeze-drying and milling.

Whereas the present invention has been described with respect to specific embodiments thereof, it will be understood that various modifications will be obvious to those skilled in the art and it is intended to encompass such modifications as fall within the scope of the appended claims.

CLAIMS:-

1. A synergistic anti-inflammatory composition including a substance containing proteins extracted from the New Zealand green-lipped mussel (*Perna canaliculus*), and one or more glycosaminoglycans.

2. The composition as claimed in claim 1 wherein the proteins extracted from the mussel are extracted from processed mussel tissue.

3. The composition as claimed in claim 2 wherein the processed mussel tissue comprises ground mussel tissue.

4. The composition as claimed in claim 2 or 3 wherein the processed mussel tissue is processed to concentrate the proteins.

5. The composition as claimed in any of claims 1 to 4 wherein the substance containing proteins extracted from the New Zealand green-lipped mussel (*Perna canaliculus*) is dried.

6 The composition as claimed in any of claims 1 to 5 wherein the substance containing proteins extracted from the New Zealand green-lipped mussel (*Perna canaliculus*) is in the form of a powder.

7. The composition as claimed in any of claims 1 to 6 wherein the glycosaminoglycan is glucosamine or a pharmaceutically acceptable salt thereof.

8. The composition as claimed in claim 7 wherein the glycosaminoglycan is glucosamine.

9. The composition as claimed in claim 7 wherein the salt is glucosamine sulphate, chondroitin sulphate or N-acetyl glucosamine.

10. A capsule including the synergistic anti-inflammatory composition as claimed in any of claims 1 to 9 wherein said composition is encapsulated in gelatine.

11. A tablet including the synergistic anti-inflammatory composition as claimed in any of claims 1 to 9.

12. A method of treating inflammation in non-human animals comprising the administration of a composition as claimed in any of claims 1 to 9.

13. A method of treating inflammation in non-human animals comprising the administration of a capsule as claimed in claim 10.

14. A method of treating inflammation in non-human animals comprising the administration of a tablet as claimed in claim 11.

15. An anti-inflammatory composition as claimed in any of claims 1 to 9 substantially as herein described.

16. A capsule as claimed in claim 10 substantially as herein described.

17. A tablet as claimed in claim 11 substantially as herein described.

18. A method of treating inflammation in non-human animals as claimed in any one of claims 12 to 14 substantially as herein described.

Patent Office

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Application No: GB 9904672.4 **Examiner:** Dr Lawrence Cullen
Claims searched: 1-18 **Date of search:** 24 May 1999

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Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.Q): A5B (BEA)

Int Cl (Ed.6): A61K 35/56, 35/60

Other: Online: CAS ONLINE, EPODOC, JAPIO, WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
A	EP 0010061 A1	(MCFARLANE) see paragraph 1, page 2 to paragraph 1, page 5.	-
A	WO 99/08535 A1	(MICROACTIVE) see lines 7-14, page 1 and Example 3	-
A	WO 96/05164	(BROADBENT) see line 10, page 2 to line 29, page 3.	-

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.